REMARKS

Applicants respectfully request entry and consideration of the amendment and remarks. Applicants submit that no issues not previously considered by the Examiner are raised by the amendment. Claims 1, 16, and 19 have been amended. Claims 1-3, 5, 6, 9, 10, and 16-21 are pending after entry of the amendment.

Applicants submit that the amended claims are supported throughout the specification, including at page 10, lines 20-25 and at page 16, Example 4.

Rejection Under 35 U.S.C. § 112, First Paragraph

The Examiner rejected claims 1-3, 5, 6, 9, 10, and 16-21 under 35 U.S.C. § 112, first paragraph, as allegedly lacking an enabling disclosure. Applicants respectfully traverse this rejection.

The Examiner asserts that the specification does not provide an enabling disclosure for growing endothelial cells in the absence of co-cultured cardiomyocytes. The Examiner asserts that cardiomyocytes are essential for achieving the intended therapeutic effect, for example, the production of cardiac like tissue and the repair of cardiac damage. Applicants do not agree.

The endothelial cells in Example 2 were not co-cultured with cardiomyocytes. Nowhere in Example 2 does it indicate that the endothelial cells were co-cultured or seeded on the alginate scaffolds with cardiomyocytes. The endothelial cells seeded onto the alginate scaffolds were from a pure population of endothelial cells expanded from endothelial cells that grew as colonies from the stripped aortic rings. See specification at page 14, lines 2-6. After 2 weeks in culture, the seeded endothelial cells were organized into a cord-like structure within the scaffold *in vitro*. See Figures 6 and 7C. Serial sections from paraffin embedded samples indicate rings composed from endothelial cells. See Figure 7C. Therefore, the data from Example 2 demonstrates (1) that endothelial cells grow and differentiate in the absence of co-cultured cardiomyocytes and (2) that cardiomyocytes are not essential for the production of aortic rings.

The Examiner asserts that the specification fails to provide any guidance as to the particular combination of factors and conditions necessary to promote the differentiation and development of embryonic or mesenchymal stem cells into cardiac myocytes or myoblasts. In the absence of specific teachings, the Examiner asserts that it would require undue

experimentation to determine the conditions under which embryonic or mesenchymal stem cells can be induced to differentiate into muscle cells *in vitro* or *in vivo*. Applicants do not agree.

Applicants submit that one skilled in the art reading this specification would be able to practice a method for repairing a damaged myocardium with mesenchymal and embryonic stem cells without undue experimentation. Applicants submit that the particular combination of factors and conditions necessary to promote differentiation of stem cells into cardiomyocytes was known to one skilled in the art. Chemicals have been reported that induce bone marrow cells into myogenic cells. Tomita et al., 1999, Circulation, 100:II247-56 at page 247, a copy of which is enclosed for the Examiner's reference. For example, bone marrow mesenchymal stem cells cultured in medium containing 5-azacytidine differentiate into cardiomyocytes and improve cardiac function when transplanted into heart tissue. Tomita et al. at page 254. Consequently, Applicants submit that based on the teachings of the specification and the art available at the time of filing of the present application, one skilled in the art could readily practice the invention without undue experimentation.

The Examiner asserts that the specification does not enable the use of all soluble growth factors. Applicants submit that the disclosure of the pore size and degradation kinetics of the microspheres would enable one skilled in the art to utilize any soluble growth factor. However, to further prosecution of the application, Applicants have restricted claims 1, 16 and 19 to "soluble angiogenic growth factors" which is fully enabled by the specification.

The Examiner asserts that the specification does not provide any guidance as to measures or methods necessary to prevent destructive allogenic or xenogenic immune responses following transplantation of the matrix containing the allogenic or xenogenic tissue. Applicants have amended the claims to require that the mammalian cells introduced onto the matrix be from the same species as the mammal in the preamble of the claim. This amendment is fully enabled by the specification.

Based on the forgoing, Applicants submit that the claims as presented are fully enabled by the specification. Withdrawal of the rejection is respectfully requested.

Summary

In view of the above amendments and the comments presented herein, favorable reconsideration in the form of a Notice of Allowance is respectfully requested. The Examiner is invited to contact Applicants' representative at 612-336-4728 if prosecution may be assisted thereby.

Respectfully submitted,

MERCHANT & GOULD P.C.
P.O. Box 2903
Minneapolis, Minnesota 55402-0903
(612) 332-5300

Dated: 12/23/02

By _____
Gregory A. Sebald
Reg. No. 33,280

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Please amend claim 1 as follows:

1. (AMENDED) A method for repairing a damaged myocardium in a mammal, comprising:

a) providing a three-dimensional porous polysaccharide matrix;

b) introducing mammalian cells from the same species into said matrix;

c) growing said cells in said matrix *in vitro*, until a tissue-engineered biograft is formed, comprising a contracting tissue; and

d) transplanting the tissue-engineered biograft onto the myocardial tissue or myocardial scar tissue of said mammal, optionally previously removing scar or dead tissue from the site of implantation;

wherein the mammalian cells are selected from the group consisting of fetal cardiomyocytes, neonatal cardiomyocytes, adult cardiac cells, fibroblasts, smooth muscle cells, endothelial cells, skeletal myoblasts, mesenchymal stem cells and embryonic stem cells; and

wherein said polysaccharide matrix further comprises controlled-release polymeric microspheres, said microspheres being capable of releasing soluble angiogenic growth factors in a controlled manner.

Please amend claims 16 as follows:

16. (AMENDED) A tissue-engineered cardiac biograft for transplantation into myocardial tissue or myocardial scar tissue, comprising:

a porous polysaccharide matrix comprising controlled-release polymeric microspheres capable of releasing soluble angiogenic growth factors; and

mammalian cells selected from the group consisting of fetal cardiomyocytes, neonatal cardiomyocytes, adult cardiac cells, fibroblasts, smooth muscle cells, endothelial cells, skeletal myoblasts, mesenchymal stem cells and embryonic stem cells;

wherein said cells have been cultured in said matrix *in vitro*.

Please amend claim 19 as follows:

19. (AMENDED) A method of preparing a three-dimensional tissue-engineered biograft comprising:

a) providing a porous polysaccharide matrix comprising microspheres capable of releasing soluble angiogenic growth factors; and

b) co-culturing the porous polysaccharide matrix *in vitro* with mammalian cells selected from the group consisting of fetal cardiomyocytes, neonatal cardiomyocytes, adult cardiac cells, fibroblasts, smooth muscle cells, endothelial cells, skeletal myoblasts, mesenchymal stem cells and embryonic stem cells, until a cardiac-like tissue is formed, comprising a tissue-engineered biograft.